STATEMENT OF WORK
Preclinical PREVENT Cancer Program: Toxicology and Pharmacology Testing

Independently and not as an agent of the Government, the Contractor shall furnish all the necessary services, qualified personnel, material, equipment, and facilities, not otherwise provided by the Government, as needed to perform the Statement of Work (SOW).

SCOPE

This contract will support the conduct of toxicological and pharmacological evaluations of potential cancer preventive agents for Investigational New Drug (IND) applications to the Food and Drug Administration (FDA) for phase 0, 1-3 clinical studies, New Drug Applications (NDA) to the FDA, and to select appropriate first-in-human (FIH) doses. This contract shall require the Contractor to perform studies in four areas: genotoxicity testing; general toxicology in experimental animals; reproductive toxicology studies in rodents and rabbits; and, specialized studies.

I. TASK AREAS

In performance of work under any Task Area the Contractor shall comply with applicable guidelines including, but not limited to, those listed in the Addendum to this Statement of Work, titled "References".

TASK AREA 1 -- GENOTOXICITY TESTING

The Contractor shall determine the homogeneity, concentration, and stability of the test substance/ test substance mixture under the conditions of use, as defined in the Task Order Statement of Work (TO SOW).

A. BACTERIAL REVERSE MUTATION TEST

The standard set of strains (±S9) shall be used, as cited in under International Conference on Harmonization (ICH) S2B Guidelines to test for G-C and A-T base changes, with positive and negative control(s). Range-finding studies shall be conducted. In some cases, other strains (e.g. nitroreductase deficient) shall be employed. Endpoints shall assess at least the following based on the cited ICH guidelines:

1. solubility
2. cytotoxicity
3. dose response
4. genotoxicity

B. AN *IN VITRO* TEST WITH CYTOGENETIC EVALUATION OF CHROMOSOMAL DAMAGE WITH MAMMALIAN CELLS

Several mammalian cell systems shall be utilized, including systems that detect gross chromosomal damage such as *in vitro* tests for structural and numerical chromosomal aberrations.

In each Task Order, the Contractor shall propose and justify an appropriate test system that is acceptable under International Conference on Harmonization (ICH) S2B Guidelines.

C. AN *IN VIVO* TEST FOR CHROMOSOMAL DAMAGE USING MOUSE OR RAT HEMATOPOIETIC CELLS

The Contractor shall conduct assays that measure either chromosomal aberrations in bone marrow cells or micronucleated polychromatic erythrocytes in bone marrow or peripheral blood.

The approach to these assays shall consider species, gender and other parameters that shall be discussed in Task Order proposals.

D. FOLLOW-UP STRATEGIES FOR POSITIVE TESTS

The Contractor shall conduct follow-up assays (e.g. RasH2 rat, Big Blue™ (lacI/cII) mouse, (+/-) p53 mouse, Comet assay, GreenScreen Human cell GADD45α-GFP, etc.) for agents with positive results in the standard genotoxicity battery.

TASK AREA 2 -- GENERAL TOXICOLOGY IN EXPERIMENTAL ANIMALS

The Contractor shall conduct studies in mice, rats and dogs. However, on rare occasions the Contractor shall use other species such as rabbits, guinea pigs, hamsters, monkeys or other animals that are deemed more appropriate due to the inherent nature of the chemopreventive agent.

The Contractor shall perform work in this Task Area as outlined in each TO SOW.

A. FOURTEEN-DAY DOSE RANGING ORAL TOXICITY IN RODENTS

The contractor shall conduct studies as follows:

1. Rats or mice of appropriate age and weight shall be procured by the Contractor from an established, reliable commercial breeder.
2. Animals shall be quarantined for one week prior to study.
3. Approximately 12 male and female rats or mice shall be used per oral dose level administered by gavage or other appropriate route determined by the Contracting Officer's Representative (COR) to obtain a range of toxic effects and mortality rates.
4. Pharmacological or toxic effects shall be recorded as to onset, duration, and disappearance over a two week period.
5. Food consumption shall be monitored and body weight shall be taken at appropriate times which will be stated in each TO SOW.
6. Survivors shall be sacrificed at the end of the observation period and gross necropsies performed on all animals.
7. Animals found dead or sacrificed in moribund condition shall be immediately necropsied or refrigerated until necropsied.
8. Tissue samples shall be taken for histopathological examination where abnormalities are noted.
9. A Lethal Dose $(LD)_{50}$ or approximate measure shall be determined along with other parameters, including but not limited to, dose range, latency, duration and reversibility of toxic effects.
10. Appropriate records as outlined in each TO SOW and according to regulatory guidelines shall be maintained on all observations, mortality, and necropsies.

B. TWENTY-EIGHT DAY STUDIES IN RODENTS AND DOGS

1. Route and timing of agent shall be by oral gavage, capsule, food admixture, or other appropriate route once per day for four weeks.
2. As appropriate the 28-day definitive study will be preceded by a 14-day range finding study to help select appropriate dosages for the 28-day study.

3. In the 28-day study, approximately 160 male and female rats or mice (20/sex/group) and 32 male and female dogs (4/sex/group) shall be chosen to provide control, low, medium, and high dosage groups.
4. If specified in the TO SOW, additional animals shall be treated in order to conduct a recovery study as well.
5. General appearance, behavior, appetite, elimination, and presence of any signs of toxicity shall be recorded daily.
6. Body weight and food consumption shall be recorded weekly with observations for mortality at least once each morning and each afternoon.
7. Clinical laboratory tests shall be performed as defined in the TO SOW.
8. All survivors shall be sacrificed during the fifth week and subjected to a detailed gross necropsy and histopathologic evaluation of major organs and tissues.
9. Histopathologic studies shall be done on high dose level and control animals and on intermediate dose level animals when there are positive findings at the high dose level.
10. The maximum tolerated and no-effect doses shall be determined and pharmacokinetic evaluations may be incorporated into these studies as defined in the TO SOW.
11. Tissues and/or fluids shall be collected for potential subsequent drug and metabolite level analyses, genomic, proteomic, metabolomic or other studies.

C. NINETY DAY, ORAL TOXICITY STUDIES IN RODENTS AND DOGS

1. Test material shall be administered daily by oral gavage, capsule, food admixture, or other appropriate route as defined in the TO SOW.
2. Approximately 100 male and 100 female rats or mice (25/dose/sex) and 32 male and female dogs (4/dose/sex) shall be used for control, low, medium, and high dosage groups.
3. Additional animals shall be treated in order to conduct a recovery study as specified in the TO SOW.
4. General appearance, behavior, appetite, elimination, and presence of any signs of toxicity shall be recorded daily.
5. Body weight and food consumption shall be recorded weekly.
6. Daily observations for mortality and weekly records of appearance, behavior, and signs of toxicologic or pharmacologic effects shall be maintained.
7. Hematological, serum chemistry, urinalysis, ophthalmology, cardiology (dogs only), and plasma drug measurements shall be made as required by TO SOW at appropriate intervals during the study.
8. All survivors shall be sacrificed after three months and subjected to detailed necropsy, including gross and microscopic evaluation.
9. All tissues from the high dose and control groups shall be microscopically evaluated and the identified target tissues shall be read in the low and medium dosage groups.
10. The maximum tolerated and no-effect doses shall be determined.
11. Tissues and/or fluids shall be collected for potential subsequent drug and metabolite level analyses, genomic, proteomic, metabolomic or other studies.

D. SIX MONTH RODENT AND NINE MONTH DOG CHRONIC TOXICITY STUDIES

1. Test material shall be administered daily by oral gavage, capsule, food admixture, or other appropriate route as defined in the TO SOW.
2. Approximately 120 male and 120 female rats or mice (30/dose/sex) and 32 male and female dogs (4/dose/sex) shall be used to provide adequate data for control, low, medium, and high dosage groups.
3. Body weight and food consumption shall be recorded weekly.
4. Daily observations for mortality and weekly records of appearance, behavior, and signs of toxicologic or pharmacologic effects shall be maintained.

5. Hematological, serum chemistry, urinalysis, ophthalmology, cardiology (dogs only), and plasma drug measurements shall be made as required by the TO SOW at appropriate intervals during the study.
6. All survivors shall be sacrificed at the end of the respective dosing periods and subjected to detailed necropsy, including gross and microscopic evaluation (high dose and control with read-down in target tissues).
7. The maximum tolerated and no-effect doses shall be determined.
8. Additional animals shall be treated in order to conduct a recovery study as specified in the TO SOW.
9. Tissues and/or fluids shall be collected for potential subsequent drug and metabolite level analyses, genomic, proteomic, metabolomic or other studies.

E. CARCINOGENICITY STUDIES IN RODENTS

As defined in the TO SOW, the Contractor shall perform the following:

1. Animals shall be six to seven weeks of age at the time of release from quarantine and start of the study.
2. All animals shall be randomized by weight and approximately sixty animals per dose per sex and species shall be started in each test group routinely.
3. Additional animals for a recovery study or interim sacrifice at 12 months shall be included as needed.
4. Sentinel animals for serological monitoring and satellite animals for blood collections shall be included as needed.
5. The test material shall be administered daily by oral gavage, capsule, food admixture, or other route.
6. Three dose levels of test agent and control groups shall be used.
7. Body weight and food consumption shall be recorded weekly.
8. Daily observations for mortality and weekly records of appearance, behavior, and signs of toxicologic or pharmacologic effects shall be maintained.
9. Hematological, serum chemistry, urinalysis, and plasma drug measurements shall be made at appropriate intervals during the study.
10. All survivors shall be sacrificed after 18 to 24 months and subjected to detailed necropsy, including gross and microscopic evaluation (high dose and control with read-down in target tissues).
11. The total spontaneous tumor incidence shall be determined and the carcinogenic potential of the test compound evaluated.
12. Tissues and/or fluids shall be collected for subsequent drug and metabolite level analyses, genomic, proteomic, metabolomic or other studies.
13. The Contractor shall also be required to conduct 6-month carcinogenicity studies in p53 +/- mice if specified in the TO SOW for the compound under study.

TASK AREA 3 -- REPRODUCTIVE TOXICITY STUDIES IN RODENTS AND RABBITS

The Contractor shall conduct studies in this Task Area using mice, rats and rabbits. However, the Contractor shall use other species when appropriate due to the inherent nature of the chemopreventive agent or a suggestion by the regulatory agencies, and could include guinea pigs, hamsters, monkeys or other animals.

The Contractor shall perform work in this Task Area as outlined in each TO SOW.

A. SEGMENT II TERATOGENICITY STUDIES

The following types of studies shall be performed to evaluate the effect of the test article on organogenesis:

1. Dose range-finding study (6 groups of 5 females).
2. Teratogenicity study (4 groups of 20 rabbits and 25 rats per group).
3. Implantations, resorptions, fetal viability and gross malformations, and soft tissue and bone malformations.

B. TWO GENERATION REPRODUCTION STUDIES IN RODENTS

The Contractor shall perform tests designed to provide information concerning effects of a test article on gonadal function, estrous cycles, mating behavior, conception, parturition, neonatal morbidity, mortality, lactation, weaning, and the growth and development of the offspring.

Depending upon the nature of the test article, second filial (F2) teratology evaluations shall be conducted as needed (F1 and F2 males shall be mated to naive females).

Endpoints include:

1. female fertility index,
2. gestation index,
3. weaning index,
4. sex ratio,
5. viability indices,
6. growth indices,
7. maternal toxicity, and
8. specialized tests, e.g., sperm, neuronal, or immune evaluations.

TASK AREA 4 -- SPECIALIZED STUDIES

The Contractor shall conduct studies in this Task Area using mice, rats, dogs, rabbits, guinea pigs, hamsters, monkeys or other appropriate species to evaluate drug-specific mechanisms of toxicity. Interspecies differences in drug metabolism, pharmacokinetics, and pharmacodynamics are well recognized and shall be considered in the development of protocols.

1. Pharmacokinetic studies shall be undertaken as needed to evaluate the absorption, plasma concentrations, and/or tissue distributions of the test article or metabolites in different formulations.
2. Deidentified human fluids, tissues, or cells (obtained commercially or from other studies) shall be employed as needed.

As outlined in Task Orders, the Contractor shall conduct studies as follows:

A. BIOANALYTICAL METHODOLOGY STUDIES

1. The Contractor shall perform bioanalytical method development and validation studies. Methodologies that shall be considered include High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), HPLC-Mass Spectroscopy (HPLC-MS), HPLC-MS-MS, GC-MS, GC-MS-MS, radioimmunoassay, immunoaffinity, atomic absorption, bioassay and other assays.
2. Analytical techniques shall be identified and validated for sample matricies with the emphasis on selectivity, accuracy, precision, lower limit of quantification, and stability as approved by the COR.

3. Isomeric composition and stability shall be considered, as necessary.
4. Formulation, plasma, blood, urine, feces, or specific tissues shall be used as necessary.
5. Applicable guidelines include, but are not limited to: Bioanalytical Method Validation Q2A; Text on Validation of Analytical Procedures; and, Q2B, Validation of Analytical Procedures: Methodology. (See the Addendum to the Statement of Work for additional guideline information.)

B. PHARMACOKINETIC AND PHARMACOKINETIC-PHARMACODYNAMIC STUDIES

The Contractor shall perform the following to determine pharmacokinetic profiles for investigational agents.

1. Pharmacokinetic studies shall provide the parameters of absorption, blood concentration-time profiles, bioavailability, distribution and elimination following administration to animals by various routes and schedules. Data on time-dependent tissue concentration of the test agent, determined as part of the toxicology testing, shall contribute to the pharmacokinetic profile.
2. Information on major metabolites shall be included in order to provide as complete a picture as possible of the overall distribution and fate of the test agent.
3. Appropriate modeling shall be applied to determine probable patterns of biodistribution and compartmentalization.
4. Radiolabeled test articles shall be used as needed. The Nuclear Regulatory Commission (NRC) licensure shall be maintained for the life of this contract.
5. Pharmacodynamic profiles (e.g. time dependent biomarker concentration or activity, effect on putative targets) shall also be determined and modeled as needed in conjunction with pharmacokinetics in order to characterize correlation of the response with drug levels and/or total exposures.
6. Determination of free drug fraction (protein unbound drug) shall be evaluated as needed.

C. BIOAVAILABILITY AND BIOEQUIVALENCE STUDIES

The Contractor shall perform bioavailability and/or bioequivalence determinations in experimental animals. Bioavailability and bioequivalence refer to absolute or relative rates and extent of an active pharmaceutical product reaching the systemic circulation or site(s) of action.

1. At least, three commonly used indices shall be estimated: the maximum drug concentration in plasma (C_{max}), the time needed to reach the maximum concentration in plasma (t_{max}), and the area under the plasma-concentration-vs.-time curve (AUC). Possible formulations/routes of administration include: oral, transdermal, subcutaneous, intramuscular, intravenous, buccal, sublingual, respiratory, vaginal, etc.
2. Effects of co-administration of other agents, dietary ingredients, or inactive ingredients shall also be evaluated as needed.
3. Isomeric composition and stability of the agents shall be considered, as necessary.
4. Radiolabeled test articles shall be used as needed and NRC licensure shall be maintained for the life of this contract.

D. DRUG METABOLISM/DRUG INTERACTION STUDIES

The Contractor shall perform studies for characterization and quantification of metabolites and/or detection and characterization of drug-drug interactions.

1. Major and active metabolites shall be identified, characterized and quantified using bioanalytical technologies such as HPLC, GC, NMR, GC-MS, GC-MS/MS, LC-MS, LC-MS/MS, immunoaffinity, etc.

2. Radiolabelled test articles shall be used as needed and NRC licensure shall be maintained for the life of this contract.
3. Isomeric composition and stability shall be considered as necessary.
4. Drug interaction experiments shall evaluate a potential for drug interactions (pharmacokinetic and/or pharmacodynamic inhibition or induction) during a combination therapy or with a likely-to-be-encountered co-medication.
5. Drug metabolism and interactions studies shall be *in vivo* (different routes of administration possible) and/or *in vitro* (e.g. microsomes, S9 fractions, hepatocytes, liver slices from experimental animal or deidentified human sources), as deemed necessary.
6. In addition to metabolic considerations, drug transporters shall also be considered, as needed.

E. SPECIFIC SAFETY STUDIES

The Contractor shall perform specialized investigations to determine mechanisms of action and toxicity of specified agents.

1. The studies shall be conducted as independent safety pharmacology studies or as a component of a larger study, such as a 90 day toxicity study. Examples of such investigations include cardiovascular toxicity, neurotoxicity, pulmonary toxicity, ototoxicity, endocrine toxicity, neurovascular toxicity, and coagulopathies.
2. Dermal and aerosol formulations/routes of administration shall be investigated, as appropriate.

F. GENOMICS/PROTEOMICS/METABOLOMICS

The Contractor shall use genomic, proteomic, metabolomic and/or other -omic tools to identify and evaluate potential for toxic reactions.

1. Genomic microarray methodology (cDNA or oligonucleotide microarrays and other related and evolving techniques) and proteomic tools [e.g., surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF); matrix-assisted laser desorption/ionization time-of-flight, (MALDI-TOF); laser capture microdissection; protein, antibody and tissue microarrays; and other related and evolving techniques] shall be used as needed.
2. These new approaches shall be used if they provide a complementary and more accurate and/or earlier assessment of toxicity risk potential, its mechanisms and targets, and lead to better-targeted animal studies using fewer animals.

G. COMPUTATIONAL (PREDICTIVE) PHARMACOLOGY/TOXICOLOGY

In silico or computational techniques shall be utilized as defined by the TO SOW to estimate solubility, intestinal permeability, metabolism, and predict toxicology (e.g. genotoxicity, hepatotoxicity, etc.) of new candidate chemopreventive agents.

H. VACCINE DEVELOPMENT STUDIES

The Contractor shall perform studies necessary to support preclinical development of an investigational vaccine.

1. *In vivo* studies shall assess immune activity, potential for local and systemic toxicity, acute and time-dependent toxicities including potential resolution. Appropriate species and end-points shall be considered in conjunction with safety assessment to assure expected immune activity and specificity. In addition, the contractor shall provide a detailed strategy for selecting and testing an appropriate adjuvant.
2. *In vitro* studies shall be conducted to assess biological or immune activity as needed.

II. GENERAL PROCEDURES

A. ANIMAL FACILITY

Laboratories shall be accredited by or registered as follows:

1. The Contractor shall have an approved Animal Welfare Assurance for the Office of Extramural Research (OER), Office of Laboratory Animal Welfare (OLAW) (http://grants.nih.gov/grants/olaw/olaw.htm), Office of the Director, NIH, as required by Section I-43-30 of the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The Contractor shall maintain such assurance for the duration of this contract, and any subcontractors performing work under this contract involving the use of animals shall also obtain and maintain an approved Animal Welfare Assurance.
2. The Contractor and any subcontractor shall be fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International or equivalent and maintain that accreditation for the life of the contract. Information about AAALAC accreditation is available at www.aaalac.org.
3. The Contractor and any subcontractor shall comply with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (http://grants.nih.gov/grants/olaw/references/phspol.htm) and conduct work in compliance with recommendations established in the Guide for the Care and Use of Laboratory Animals (http://www.nap.edu/openbook.php?record_id=5140).
4. The Contractor's Institutional Animal Care and Use Committee (IACUC) shall approve all animal procedures under this contract.

All animals for toxicological studies shall be furnished by the Contractor from established, reputable, known commercial breeders. The Contractor shall quarantine the animals for an appropriate period prior to placing them on test for toxicological studies and their release shall be documented by a veterinarian certified by the American College of Laboratory Animal Medicine (ACLAM).

B. GOOD LABORATORY PRACTICE (GLP) REGULATIONS

The Contractor shall develop experimental protocols and conduct studies using established techniques, study parameters, and statistical methods of data analysis in accordance with current FDA GLP regulations for non clinical laboratory studies (21 CFR Part 58) as published in the Friday, December 22, 1978 Federal Register, Vol. 43, No. 247, pp. 59986-60025. (http://www.access.gpo.gov/nara/cfr/waisidx_01/21 cfr58_01.html) and International Conference on Harmonization (ICH) Guidelines (http://www.fda.gov/cder/guidance/959fnl.pdf). The format and content of the technical report shall conform to current FDA GLP standards. (See Adendum to the Statement of Work)

The Contractor shall possess and maintain knowledge of FDA GLP regulations in order to be able to conduct and report these studies accordingly. The Contractor shall ensure compliance with current GLP regulations on all studies, and provide documentation to the COR of any FDA data audits and inspections (i.e., reports and responses to issues raised) within one (1) week after receipt of the audit or inspection report from the FDA. If it is determined that problems are encountered with the assays or documentation because GLP conditions were not met or maintained, the Contractor shall repeat the assay at no additional cost to the Government.

C. PATHOLOGY

The Contractor shall ensure that examination and reporting of pathologic alterations in organs and

tissues in each toxicological study are conducted to demonstrate histopathological evaluations correlated to clinical, hematologic, or clinical chemistry evidence of toxicity to an organ system with direct injury to that system. The Contractor shall accurately report the type and severity of lesions in animals in order to increase the efficiency of prediction for toxic manifestations of agents administered to patients in subsequent clinical trials.

The Contractor shall ensure that:

1. All lesions are categorized as either drug-related or non drug-related;
2. Each lesion is listed and coded by the most specific topographical and morphological diagnoses, severity, and distribution using Systemized Nomenclature of Medicine (SNOMED) codes.

Pathology examinations and procedures shall include at least the following, as appropriate and as specified in each Task Order SOW:

Rats:

1. A complete necropsy, organ weight determinations, clinical chemistry, hematology, coagulation, and histopathology shall be performed on treated and control animals.
2. Animals shall be observed two times daily (once in the morning and once in the afternoon at least 4 hours apart, including holidays and weekends) for moribundity and mortality; one of these observation periods shall be shortly after dosing to observe potential effects of dosing
3. Gross motor and behavioral activity, observable changes in appearance, and clinical signs related to the pharmacology and toxicology of the test article shall be recorded with attention to the sign, onset, and duration.
4. Animals whose condition makes it unlikely that they will survive until the next observation, based upon criteria established by the Contractor's Principal Investigator in concert with the veterinary staff and toxicologist, shall be sacrificed immediately, necropsied, and diagnosed histopathologically (unless the cause of death is grossly related to the gavage procedure). Moribund animals shall be terminated out of sequence with complete necropsy and histopathology as for scheduled necropsies.
5. Antemortem observations shall be recorded for each animal prior to necropsy, including those clinically normal.
6. All significant antemortem observations shall receive comment or confirmation at necropsy. All animals shall have final body weights and required organ weights taken, unless severely autolyzed.
7. A pathologist shall be available to examine any unusual findings during unscheduled necropsies and shall be present at the terminal sacrifices.
8. The Contractor shall obtain and analyze additional samples during severe or unusual toxicity (e.g. plasma samples for clinical pathology/clinical chemistry/coagulation/plasma drug levels) and/or modify doses of the agents, If severe or unusual toxicity is noted, these additional plasma samples shall be obtained at the onset of the observation and the Contracting Officer's Representative (COR) shall be notified within 24 hours.

    a. Histopathological observations shall be made on all required tissues of animals in the control group and in the highest combination treated group that has greater than 50% survival.
    b. Tissues from animals in other groups shall be fixed and held for possible further study.
    c. Tissues from organs found to be normal in a higher dose groups need not be examined histologically in lower dose groups.
    d. Tissues from organs which are found to be abnormal in a higher dose group shall be examined in animals from the other groups.

9. Tissues that shall be examined macroscopically in all animals and microscopically in controls and

the above-specified animals include, but are not limited to: (*record weight of tissues):

adrenals (pair) aorta (thoracic) bladder (urinary) bone (sternum) and marrow (femur) *brain (3 levels) cecum colon corpus and cervix uteri duodenum epididymis esophagus eyes *heart ileum	jejunum *kidneys *liver lungs and bronchi (infused with formalin) lymph node (mesenteric) mammary gland ovaries and fallopian tubes pancreas pituitary prostate salivary gland sciatic nerve seminal vesicle skeletal muscle	skin spinal cord (2 levels; entire cord if signs indicate cord involvement) *spleen stomach *testes thymus *thyroid/parathyroid trachea urinary bladder vagina lesions (all gross visible) *tissue masses abnormal lymph nodes

10. Bone marrow smears shall be prepared, inventoried, and properly stored until shipped to the DCP Chemoprevention Repository at the time of Task Order close-out.
11. Teratological examinations of rodents, when needed, shall be similar to that described in the section on Rabbits below.
12. The Draft and Final report of each study shall contain high resolution, illustrative, photographs or digital images of drug-induced lesions.

Dogs:

1. A complete necropsy, organ weight determinations, and histopathology shall be performed on treated and control animals.
2. Dogs shall be checked for mortality and signs of morbidity twice daily, as noted above. Moribund dogs shall be terminated out of sequence with complete necropsy and histopathology as for scheduled necropsies.
3. Antemortem observations shall be recorded for each dog prior to necropsy, including those clinically normal.
4. All significant antemortem observations shall receive comment or confirmation at necropsy.
5. All dogs shall have final body weights and required organ weights taken, unless severely autolyzed.
6. A pathologist shall be available to examine any unusual findings during unscheduled necropsies and shall be present at the terminal sacrifices.

7. The Contractor shall obtain and analyze additional samples during severe or unusual toxicity (e.g. plasma samples for clinical pathology/clinical chemistry/coagulation/plasma drug levels) and/or modify doses of the agents. If severe or unusual toxicity is noted, these additional plasma samples shall be obtained at the onset of the observation and the COR shall be notified within 24 hours.

    a. Histopathologic observations shall be made on all required tissues of dogs in the control group and in the highest combination treated group that has greater than 50% survival.
    b. Tissues from dogs in other groups shall be fixed and held for possible further study.

c. Tissues from organs found to be normal in a higher dose group need not be examined histologically in lower dose groups.
d. Tissues from organs which are found to be abnormal in a higher dose group shall be examined in dogs from the lower dose groups.

8. Tissues that shall be examined macroscopically in all animals and microscopically in controls and the above-specified dogs include, but are not limited to: (*record weights of tissues):

adrenals (pair) aorta (thoracic) bone (femur with epiphyseal plate of head) bone marrow (sternum) *brain (3 levels) cecum colon corpus and cervix uteri duodenum (incl. bile and pancreatic ducts) epididymis esophagus eyes gall bladder heart ileum jejunum *kidneys (weigh separately) *liver (weigh total right	medial lobe with section of gall bladder and left lateral lobe) lungs and bronchi (left atypical and left diaphragmatic lobes; infuse one lobe with formalin; sample both infused and non-infused tissues lymph node (bronchial, mandibular, mesenteric) mammary gland (from both sexes) ovaries and fallopian tubes pancreas (head and tail) pituitary prostate rectum salivary gland (mandibular) sciatic nerve (longitudinal section from region of proximal femur) skeletal muscle skin (nonfrictional surface, dorsal thorax; frictional site, elbow)	spinal cord (2 levels; entire cord if signs indicate cord involvement) *spleen stomach *testes *thymus *thyroid/parathyroid (record combined weight) tongue tonsil trachea ureter urinary bladder vagina and all gross visible lesions *tissue masses abnormal lymph nodes

9. Bone marrow smears shall be prepared, inventoried and properly stored until shipment to the Chemoprevention Repository at the time of contract close-out.
10. The Draft and Final reports for each study shall contain high resolution, illustrative, photographs or digital images of all drug-induced lesions.

Rabbits:

The Contractor shall describe criteria for determining the viability of a fetus, early and late resorptions, and for the detection of possible implantations sites, and use 10% ammonium sulfide in the apparently nongravid uterus.

1. In teratology studies, animals shall be sacrificed on day 20 of presumed gestation.
2. A Caesarian section shall be performed immediately upon sacrifice.

3. The abdominal and thoracic cavities shall be opened and examined.
4. The ovaries of all animals (gravid and apparently nongravid) shall be examined and the number of corpora lutea on each ovary recorded.
5. The uterus of gravid animals shall be examined and weighed.
6. The number and location of fetuses and early and late resorptions shall be recorded.
7. Analyses of the uterus shall include, at least, by absolute number and percent:

    a. Corpora lutea (CL) and implantations per dam
    b. Percent pre- and post- implantation loss
    c. Live and dead fetuses per litter
    d. Resorptions (early and late) per litter
    e. Litter with resorptions, litters with total resorptions
    f. Nonlive[1] per litter, litters with nonlive
    g. Affected[2] per litter, litters with affected

    [1] nonlive = dead + resorbed

    [2] affected = nonlive + externally malformed

8. Fetal observations shall include individual body weight and sex.
9. All fetuses shall be examined externally and findings recorded.
10. Fetuses in the range finding study with gross external alterations shall be preserved in Bouin's solution for possible future examination.

11. Analyses shall include, at least, by absolute number and percent:

    a. Live fetuses per litter, litters with live fetuses
    b. Sex ratio per litter Mean fetal body weight by litter and by sex within litter
    c. Externally malformed fetuses per litter
    d. Litters with externally malformed fetuses
    e. Externally malformed fetuses by sex per litter

12. A morphological examination of the fetuses shall be performed and include a detailed evaluation of the eyes, palate, head shape, and trunk and extremities.
13. Abnormal findings shall be recorded.
14. In Segment II teratology studies, but not in range finding studies, each fetus shall be further identified by litter and uterine placement, and one-half of the viable and nonviable fetuses shall be fixed in Bouin's solution and viscerally examined using the Wilson's free-hand slicing technique.
15. Remaining fetuses shall be evascerated and the skeletons stained with Alizarin Red S, examined for alterations, cleared in glycerin, and stored in 99.5% glycerin / 0.5% phenol.
16. All abnormal findings shall be recorded.
17. Analyses shall further include all the above and, at least, by absolute number and percent:

    a. Fetuses malformed externally, skeletally, and viscerally by litter
    b. Litters with malformed fetuses
    c. Malformed fetuses by litter and by sex within litter
    d. Types and incidence of individual malformations
    e. Fetuses and their malformations by litter and dose
    f. Fetuses with variations per litter
    g. Litters with fetuses having variations
    h. Types and incidences of individual variations
    i. Crown to rump length

18. The Draft and Final reports for each study shall contain high resolution, illustrative, photographs

or digital images of drug-induced malformations.

Monkeys:

Depending upon the study design specified in each Task Order, histopathological examination shall be as extensive as that specified for dog studies above. In specialized studies involving monkeys, or other species, only specific pharmacokinetic and/or pharmacodynamic endpoints shall be evaluated as determined by the COR.

D. ASSAY OF TEST MATERIAL AND FORMULATIONS

1. NCI staff shall provide cancer chemoprevention agents (chemical or biological, including vaccines and antibodies) in suitable quantity and quality. Generally a Certificate of Analysis, Material Safety Data Sheet, and confirmation of identity will be provided with the agent; in some instances, the latter shall be required to be performed by the Contractor as directed in the TO SOW.
2. The Contractor shall determine the purity of the neat compound before and after the in-life study, as well as the homogeneity, concentration, and stability of the formulated material under the conditions of use.
3. The Contractor shall use analytical instrumentation to develop, refine, and adapt the methods (e.g., for plasma or other matrices).
4. Dose accuracy shall be ± 10% of theoretical.
5. Dose concentration shall be adjusted to accommodate animal weight changes.
6. Although most studies shall be conducted using oral gavage to rats and capsules to dogs, other routes such as admixture with food, dissolution into drinking water, dermal, aerosol, etc. may be appropriate.

E. OTHER CONSIDERATIONS

1. Protocol Modifications:

    a. Additions, modifications or deletions of protocols shall be approved by the COR prior to implementation.
    b. If unexpected adverse events are observed at any stage of evaluation that would jeopardize the progress of a study, the Contracotor shall contact the COR as early as possible but within 24 hours to report the findings.
    c. If a test fails to provide the information needed for example as a result of misdosing of animals, errors in formulation, unapproved protocol deviations, loss of data, or non-Good Laboratory Practice (non-GLP) performance then the protocol shall be repeated at the direction of the COR and at the expense of the Contractor.
    d. The rerun may be modified within the original scope of work as agreed by the COR.

2. Quality Assurance Unit:

    a. Studies shall be audited for GLP compliance by a Quality Assurance Unit.
    b. The Final report for each study shall include documentation and state that the study was conducted under GLP.

3. Statistics:

    a. The Contractor's historical in-house and current referenced data bases shall be used as a basis for the underlying normal distribution and appropriate statistical tests (parametric versus nonparametric).

b. In addition to analysis of variance (ANOVA) and t-tests, linear regression analysis shall be performed, all using validated computer system(s).
c. The Final report for each study shall include a description of the statistical analyses used.

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TASK AREA 2 - GENERAL TOXICOLOGY IN EXPERIMENTAL ANIMALS

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TASK AREA 3 - REPRODUCTIVE TOXICITY STUDIES IN RODENTS AND RABBITS

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Environmental Protection Agency. Guidelines for Developmental Toxicity Risk Assessment. 1991 Dec. Available from: http://www.epa.gov/raf/publications/pdfs/DEVTOX.PDF

TASK AREA 4 - SPECIALIZED STUDIES

FDA. Guidance for Industry: M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, Questions and Answers (R2). 2013 Feb. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292340.pdf

i. BIOANALYTICAL METHODOLOGY STUDIES

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ii. PHARMACOKINETIC AND PHARMACOKINETIC-PHARMACODYNAMIC STUDIES

FDA. Guideline for Industry: S3A Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies. 1995 Mar. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm074937.pdf

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iii. BIOAVAILABILITY AND BIOEQUIVALENCE STUDIES

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v. SPECIFIC SAFETY STUDIES

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